

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claim 1 (currently amended): A method for detecting the presence or absence of a fetal chromosomal abnormality, said method comprising: quantitating the relative amount of the alleles at a heterozygous locus of interest, wherein said heterozygous locus of interest ~~[[was]]~~has been identified by determining the sequence of alleles at a locus of interest from template DNA obtained from a sample from a pregnant female, wherein said relative amount is expressed as a ratio, ~~[[and]]~~ wherein said ratio indicates the presence or absence of a fetal chromosomal abnormality, and wherein said template DNA comprises a mixture of maternal DNA and fetal DNA.

Claim 2 (original): The method of claim 1, wherein said template DNA is obtained from a source selected from the group consisting of human, non-human, mammal, reptile, cattle, cat, dog, goat, swine, pig, monkey, ape, gorilla, bull, cow, bear, horse, sheep, poultry, mouse, rat, fish, dolphin, whale, and shark.

Claim 3 (original): The method of claim 2, wherein the template DNA is obtained from a human source.

Claim 4 (currently amended): The method of claim 1, wherein the template DNA is obtained from a sample selected from the group consisting of: a ~~cell, fetal cell, tissue,~~ blood, serum, plasma, saliva, urine, tear, vaginal secretion, ~~umbilical cord blood, chorionic villi, amniotic fluid, embryonic tissue, an embryo, a four-celled embryo, an eight-celled embryo, a 16-celled embryo, a 32-celled embryo, a 64-celled embryo, a 128-celled embryo, a 256-celled embryo, a 512-celled embryo, a 1024-celled embryo,~~ lymph fluid, cerebrospinal fluid, mucosa secretion, peritoneal fluid, ascitic fluid, fecal matter, and body exudates.

Claim 5 (original): The method of claim 1, wherein alleles of multiple loci of interest are sequenced and their relative amounts quantitated and expressed as a ratio.

Claim 6 (original): The method of claim 5, wherein said multiple loci of interest are on multiple chromosomes.

Claim 7 (cancelled)

Claim 8 (currently amended): The method of claim ~~[[7]]~~3, wherein template DNA from said pregnant female is obtained from a sample selected from the group consisting of: ~~cells, tissues,~~ blood, serum, plasma, saliva, urine, tear, vaginal secretion, lymph fluid, cerebrospinal fluid, mucosa secretion, peritoneal fluid, ascitic fluid, fecal matter, ~~umbilical cord blood, chorionic villi, amniotic fluid and~~ body exudate.

Claim 9 (original): The method of claim 4, wherein said sample is mixed with an agent that inhibits cell lysis to inhibit the lysis of cells, if cells are present, wherein the agent is selected from the group consisting of membrane stabilizer, cross-linker, and cell lysis inhibitor.

Claim 10 (currently amended): The method of claim 9 wherein said agent is a cell lysis inhibitor.

Claim 11 (original): The method of claim 10, wherein said cell lysis inhibitor is selected from the group consisting of glutaraldehyde, derivatives of glutaraldehyde, formaldehyde, formalin, and derivatives of formaldehyde.

Claim 12 (original): The method of claim 9, wherein said sample is blood.

Claim 13 (cancelled)

Claim 14 (currently amended): The method of claim ~~[[13]]~~12, wherein said blood is obtained from a human pregnant female when the fetus is at a gestational age selected from the

group consisting of: 0-4, 4-8, 8-12, 12-16, 16-20, 20-24, 24-28, 28-32, 32-36, 36-40, 40-44, 44-48, 48-52, and more than 52 weeks.

Claim 15 (currently amended): The method of claim ~~[[13]]~~12, wherein said template DNA is obtained from plasma from said blood.

Claim 16 (currently amended): The method of claim ~~[[13]]~~12, wherein said template DNA is obtained from serum from said blood.

Claim 17 (cancelled)

Claim 18 (currently amended): The method of claim ~~[[17]]~~15 or 16, wherein prior to determining the sequence of alleles of a locus of interest from template DNA, maternal DNA is sequenced to identify a homozygous locus of interest, and further wherein said homozygous locus of interest is the locus of interest analyzed in the template DNA.

Claim 19 (currently amended): The method of claim ~~[[17]]~~15 or 16, wherein prior to determining the sequence of alleles of a locus of interest from template DNA, maternal DNA is sequenced to identify a heterozygous locus of interest, and further wherein said heterozygous locus of interest is the locus of interest analyzed in the template DNA.

Claim 20 (original): The method of claim 1, wherein determining the sequence of the alleles comprises:

- (a) amplifying alleles of a locus of interest on a template DNA using a first and a second primer, wherein the second primer contains a recognition site for a restriction enzyme such that digestion with the restriction enzyme generates a 5' overhang containing the locus of interest;
- (b) digesting the amplified DNA with the restriction enzyme that recognizes the recognition site on the second primer;

- (c) incorporating a nucleotide into the digested DNA of (b) by using the 5' overhang containing the locus of interest as a template; and
- (d) determining the sequence of the alleles of the locus of interest by determining the sequence of the DNA of (c).

Claim 21 (original): The method of claim 1, wherein determining the sequence of alleles comprises:

- (a) amplifying alleles of a locus of interest on a template DNA using a first and second primers, wherein the second primer contains a recognition site for a restriction enzyme such that digestion with the restriction enzyme generates a 5' overhang containing the locus of interest;
- (b) digesting the amplified DNA with the restriction enzyme that recognizes the recognition site on the second primer;
- (c) incorporating nucleotides into the digested DNA of (b), wherein;
 - (i) a nucleotide that terminates elongation, and is complementary to the locus of interest of an allele, is incorporated into the 5' overhang of said allele, and
 - (ii) a nucleotide complementary to the locus of interest of a different allele is incorporated into the 5' overhang of said different allele, and said terminating nucleotide, which is complementary to a nucleotide in the 5' overhang of said different allele, is incorporated into the 5' overhang of said different allele.
- (d) determining the sequence of the alleles of a locus of interest by determining the sequence of the DNA of (c).

Claim 22 (original): The method of claim 20 or 21, wherein the incorporation of a nucleotide in (c) is by a DNA polymerase selected from the group consisting of E. coli DNA polymerase, Klenow fragment of E. coli DNA polymerase I, T7 DNA polymerase, T4 DNA polymerase, Taq polymerase, Pfu DNA polymerase, Vent DNA polymerase and sequenase.

Claim 23 (original): The method of claim 20, wherein the incorporation of a nucleotide in (c) comprises incorporation of a labeled nucleotide.

Claim 24 (original): The method of claim 20, wherein the incorporation of a nucleotide in (c) comprises incorporation of a dideoxynucleotide.

Claim 25 (original): The method of claim 20, wherein the incorporation of a nucleotide in (c) further-comprises incorporation of a deoxynucleotide and a dideoxynucleotide.

Claim 26 (original): The method of claim 1, wherein the incorporation of a nucleotide in (c) further comprises using a mixture of labeled and unlabeled nucleotides.

Claim 27 (original): The method of claim 23, wherein the labeled nucleotide is labeled with a molecule selected from the group consisting of radioactive molecule, fluorescent molecule, antibody, antibody fragment, hapten, carbohydrate, biotin, derivative of biotin, phosphorescent moiety, luminescent moiety, electrochemiluminescent moiety, chromatic moiety, and moiety having a detectable electron spin resonance, electrical capacitance, dielectric constant and electrical conductivity.

Claim 28 (original): The method of claim 27, wherein the labeled nucleotide is labeled with a fluorescent molecule.

Claim 29 (original): The method of claim 21, wherein the incorporation of a nucleotide in (c)(i) comprises incorporation of a labeled nucleotide.

Claim 30 (original): The method of claim 21, wherein the incorporation of a nucleotide in (c)(i) comprises incorporation of a dideoxynucleotide.

Claim 31 (original): The method of claim 21, wherein the incorporation of a nucleotide in (c)(i) further comprises incorporation of a deoxynucleotide and a dideoxynucleotide.

Claim 32 (original): The method of claim 21, wherein the incorporation of a nucleotide in (c)(i) further comprises using a mixture of labeled and unlabeled nucleotides.

Claim 33 (original): The method of claim 21, wherein the incorporation of a nucleotide in (c)(ii) comprises incorporation of a labeled nucleotide.

Claim 34 (original): The method of claim 21, wherein the incorporation of a nucleotide in (c)(ii) comprises incorporation of a deoxynucleotide.

Claim 35 (original): The method of claim 21, wherein the incorporation of a nucleotide in (c)(ii) further comprises incorporation of a deoxynucleotide and a dideoxynucleotide.

Claim 36 (original): The method of claim 21, wherein the incorporation of a nucleotide in (c)(ii) further comprises using a mixture of labeled and unlabeled nucleotides.

Claim 37 (original): The method of claim 29, wherein the labeled nucleotide is a dideoxynucleotide.

Claim 38 (original): The method of claim 29, wherein the labeled nucleotide is labeled with a molecule selected from the group consisting of radioactive molecule, fluorescent molecule, antibody, antibody fragment, hapten, carbohydrate, biotin, derivative of biotin, phosphorescent moiety, luminescent moiety, electrochemiluminescent moiety, chromatic moiety, and moiety having a detectable electron spin resonance, electrical capacitance, dielectric constant and electrical conductivity.

Claim 39 (original): The method of claim 38, wherein the labeled nucleotide is labeled with a fluorescent molecule.

Claim 40 (original): The method of claim 39, wherein the incorporation of a nucleotide in (c)(i) further comprises incorporation of an unlabeled nucleotide.

Claim 41 (original): The method of claim 20 or 21, wherein the determination of the sequence of the locus of interest in (d) comprises detecting a nucleotide.

Claim 42 (original): The method of claim 20 or 21, wherein said first and second primers contain a portion of a restriction enzyme recognition site that contains a variable nucleotide, wherein the full restriction enzyme recognition site is generated after amplification.

Claim 43 (original): The method of claim 20 or 21, wherein the restriction enzyme recognition site is for a restriction enzyme selected from the group consisting of BsaJ I, Bssk I, Dde I, EcoN I, Fnu4H I, Hinf I, and ScrF I.

Claim 44 (original): The method of claim 20 or 21, wherein the restriction enzyme cuts DNA at a distance from the recognition site.

Claim 45 (original): The method of claim 44, wherein the recognition site is for a Type IIS restriction enzyme.

Claim 46 (original): The method of claim 45, wherein the Type IIS restriction enzyme is selected from the group consisting of: Alw I, Alw26 I, Bbs I, Bbv I, BceA I, Bmr I, Bsa I, Bst71 I, BsmA I, BsmB I, BsmF I, BspM I, Ear I, Fau I, Fok I, Hga I, Ple I, Sap I, SSfaN I, and Sthi32 I.

Claim 47 (original): The method of claim 20 or 21, wherein said method of amplification is selected from the group consisting of: polymerase chain reaction, self-sustained sequence reaction,

ligase chain reaction, rapid amplification of cDNA ends, polymerase chain reaction and ligase chain reaction, Q-beta phage amplification, strand displacement amplification, and splice overlap extension polymerase chain reaction.

Claim 48 (original): The method of claim 47, wherein said method of amplification is PCR.

Claim 49 (original): The method of claim 48, wherein an annealing temperature for cycle 1 of PCR is about the melting temperature of the portion of the 3' region of the second primer that anneals to the template DNA.

Claim 50 (original): The method of claim 49, wherein an annealing temperature for cycle 2 of PCR is about the melting temperature of the portion of the 3' region of the first primer that anneals to the template DNA.

Claim 51 (original): The method of claim 50, wherein an annealing temperature for the remaining cycles of PCR is at about the melting temperature of the entire second primer.

Claim 52 (currently amended): The method of claim 1, wherein determining the sequence comprises a method selected from the group consisting of: allele specific PCR, mass spectrometry, hybridization, primer extension, fluorescence resonance energy transfer (FRET), sequencing, Sanger dideoxy sequencing, DNA microarray, ~~GeneCHIP arrays, HuSNP arrays, CodeLink Arrays, BeadArray Technology, MassARRAY, MassEXTEND, SNP-IT, TaqMan, InvaderStrand Assay,~~ southern blot, slot blot, dot blot, and MALDI-TOF mass spectrometry.

Claim 53 (original): The method of claim 1, wherein said ratio for alleles at heterozygous loci of interest on a chromosome are summed and compared to the ratio for alleles at heterozygous loci of interest on a different chromosome, wherein a difference in ratios indicates the presence of a chromosomal abnormality.

Claim 54 (original): The method of claim 53, wherein the chromosomes that are compared are human chromosomes selected from the group consisting of: chromosome 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, X, and Y.

Claim 55 (original): The method of claim 53, wherein the ratio for the alleles at heterozygous loci of interest of chromosomes 13, 18, and 21 are compared.

Claim 56 (original): The method of claim 1, wherein said locus of interest is a single nucleotide polymorphism.

Claim 57 (original): The method of claim 1, wherein said locus of interest is a mutation.

Claim 58 (currently amended): A method comprising determining the sequence of a locus of interest on free fetal DNA from a sample comprising free fetal DNA, wherein an agent that inhibits cell lysis has been added to said sample to inhibit lysis of cells, if cells are present, wherein said agent is selected from the group consisting of membrane stabilizer, cross-linker, and cell lysis inhibitor.

Claim 59 (currently amended): The method of claim 58, wherein said sample is selected from the group consisting of: ~~tissue, cell,~~ blood, serum, plasma, urine, and vaginal secretion.

Claim 60 (original): The method of claim 59, wherein said sample is blood.

Claim 61 (original): The method of claim 58, wherein said sample comprises free maternal template DNA and free fetal template DNA.

Claim 62 (original): The method of claim 58, wherein said agent is a cell lysis inhibitor.

Claim 63 (original): The method of claim 62, wherein said cell lysis inhibitor is selected from the group consisting of: glutaraldehyde, derivatives of glutaraldehyde, formaldehyde, derivatives of formaldehyde, and formalin.

Claim 64 (original): The method of claim 58, wherein prior to determining the sequence, template DNA was isolated.

Claim 65 (original): The method of claim 60, wherein said template DNA is obtained from plasma of said blood.

Claim 66 (original): The method of claim 60, wherein said template DNA is obtained from serum of said blood.

Claim 67 (original): The method of claim 58, wherein prior to determining the sequence of the locus of interest on fetal DNA, the sequence of the locus of interest on maternal template DNA was determined.

Claim 68 (original): The method of claim 58, wherein prior to determining the sequence of the locus of interest on fetal DNA, the sequence of the locus of interest on paternal template DNA was determined.

Claim 69 (original): The method of claim 58, wherein said locus of interest is a single nucleotide polymorphism.

Claim 70 (original): The method of claim 58, wherein said locus of interest is a mutation.

Claim 71 (original): The method of claim 58, wherein the sequence of multiple loci of interest is determined.

Claim 72 (original): The method of claim 71, wherein the multiple loci of interest are on multiple chromosomes.

Claim 73 (original): The method of claim 58, wherein determining the sequence comprises:

- (a) amplifying a locus of interest on a template DNA using a first and second primers, wherein the second primer contains a recognition site for a restriction enzyme such that digestion with the restriction enzyme generates a 5' overhang containing the locus of interest;
- (b) digesting the amplified DNA with the restriction enzyme that recognizes the recognition site on the second primer;
- (c) incorporating a nucleotide into the digested DNA of (b) by using the 5' overhang containing the locus of interest as a template; and
- (d) determining the sequence of the locus of interest by determining the sequence of the DNA of (c).

Claim 74 (original): The method of claim 58, wherein determining the sequence comprises:

- (a) amplifying alleles of a locus of interest on a template DNA using a first and second primers, wherein the second primer contains a recognition site for a restriction enzyme such that digestion with the restriction enzyme generates a 5' overhang containing the locus of interest;
- (b) digesting the amplified DNA with the restriction enzyme that recognizes the recognition site on the second primer;
- (c) incorporating nucleotides into the digested DNA of (b), wherein:
 - (i) a nucleotide that terminates elongation, and is complementary to the locus of interest of an allele, is incorporated into the 5' overhang of said allele, and
 - (ii) a nucleotide complementary to the locus of interest of a different allele is incorporated into the 5' overhang of said different allele, and said terminating nucleotide, which is complementary to a nucleotide in the 5' overhang of said different allele, is incorporated into the 5' overhang of said different allele.

(d) determining the sequence of the alleles of a locus of interest by determining the sequence of the DNA of (c).

Claim 75 (original): The method of claim 73 or 74, wherein the restriction enzyme cuts DNA at a distance from the recognition site.

Claim 76 (original): The method of claim 75, wherein the recognition site is for a Type IIS restriction enzyme.

Claim 77 (original): The method of claim 76, wherein the Type IIS restriction enzyme is selected from the group consisting of: Alw I, Alw26 I, Bbs I, Bbv I, BceA I, Bmr I, Bsa I, Bst71 I, BsmA I, BsmB I, BsmF I, BspM I, Ear I, Fau I, Fok I, Hga I, Ple I, Sap I, SSfaN I, and Sthi32 I.

Claim 78 (original): The method of claim 73 or 74, wherein said method of amplification is selected from the group consisting of: polymerase chain reaction, self-sustained sequence reaction, ligase chain reaction, rapid amplification of cDNA ends, polymerase chain reaction and ligase chain reaction, Q-beta phage amplification, strand displacement amplification, and splice overlap extension polymerase chain reaction.

Claim 79 (original): The method of claim 78, wherein said method of amplification is by PCR.

Claim 80 (original): The method of claim 79, wherein an annealing temperature for cycle 1 of PCR is about the melting temperature of the portion of the 3' region of the second primer that anneals to the template DNA.

Claim 81 (original): The method of claim 80, wherein an annealing temperature for cycle 2 of PCR is about the melting temperature of the portion of the 3' region of the first primer that anneals to the template DNA.

Claim 82 (original): The method of claim 81, wherein an annealing temperature for the remaining cycles of PCR is at about the melting temperature of the entire second primer.

Claim 83 (currently amended): The method of claim 58, wherein the sequence of a locus of interest was determined using a method selected from the group consisting of: allele specific PCR, mass spectrometry, hybridization, primer extension, fluorescence polarization, fluorescence resonance energy transfer (FRET), fluorescence detection, sequencing, Sanger dideoxy sequencing, DNA ~~micorarray~~ microarray, southern blot, slot blot, dot blot, and MALDI-TOF mass spectrometry.

Claim 84 (currently amended): A method for determining the sequence of a locus of interest ~~in a sample comprising fetal DNA~~ from template DNA, wherein the template DNA comprises a mixture of fetal DNA and maternal DNA and is obtained from a sample from a pregnant female, said method comprising:

- (a) amplifying a locus of interest on ~~[[a]]~~ the template DNA using a first and second primers, wherein the second primer contains a recognition site for a restriction enzyme such that digestion with the restriction enzyme generates a 5' overhang containing the locus of interest;
- (b) digesting the amplified DNA with the restriction enzyme that recognizes the recognition site on the second primer;
- (c) incorporating a nucleotide into the digested DNA of (b) by using the 5' overhang containing the locus of interest as a template; and
- (d) determining the sequence of the locus of interest by determining the sequence of the DNA of (c).

Claim 85 (currently amended): A method for determining the sequence of alleles of a locus of interest ~~in a sample comprising fetal DNA~~ from template DNA, wherein the template DNA comprises a mixture of fetal DNA and maternal DNA and is obtained from a sample from a pregnant female, said method comprising:

- (a) amplifying alleles of a locus of interest on a template DNA using a first and second primers, wherein the second primer contains a recognition site for a restriction enzyme such that digestion with the restriction enzyme generates a 5' overhang containing the locus of interest;
- (b) digesting the amplified DNA with the restriction enzyme that recognizes the recognition site on the second primer;
- (c) incorporating nucleotides into the digested DNA of (b), wherein:
 - (i) a nucleotide that terminates elongation, and is complementary to the locus of interest of an allele, is incorporated into the 5' overhang of said allele, and
 - (ii) a nucleotide complementary to the locus of interest of a different allele is incorporated into the 5' overhang of said different allele, and said terminating nucleotide, which is complementary to a nucleotide in the 5' overhang of said different allele, is incorporated into the 5' overhang of said different allele;
- (d) determining the sequence of the alleles of a locus of interest by determining the sequence of the DNA of (c).

Claim 86 (currently amended): The method of claim 84 or claim 85, wherein said sample is selected from the group consisting of ~~cell, tissue, blood, serum, plasma, saliva, urine, tears, vaginal secretion, sweat, umbilical cord blood, chorionic villi, amniotic fluid, embryonic tissue, embryo, a two-celled embryo, a four-celled embryo, an eight-celled embryo, a 16-celled embryo, a 32-celled embryo, a 64-celled embryo, a 128-celled embryo, a 256-celled embryo, a 512-celled embryo, a 1024-celled embryo,~~ lymph fluid, cerebrospinal fluid, mucosa secretion, peritoneal fluid, ascitic fluid, fecal matter, and body exudates.

Claim 87 (currently amended): A method for preparing a sample for analysis comprising isolating free nucleic acid from a sample that contains nucleic acid, wherein an agent that inhibits cell lysis ~~inhibitor~~ has been added to the sample to inhibit lysis of cells, if cells are present, and wherein said agent is selected from the group consisting of membrane stabilizer, cross-linker, and cell lysis inhibitor.

Claim 88 (original): The method of claim 87, wherein said sample is obtained from a source selected from the group consisting of human, non-human, mammal, reptile, cattle, cat, dog, goat, swine, pig, monkey, ape, gorilla, bull, cow, bear, horse, sheep, poultry, mouse, rat, fish, dolphin, whale, and shark.

Claim 89 (original): The method of claim 88, wherein the sample is obtained from a human source.

Claim 90 (currently amended): The method of claim 87, wherein the sample is obtained from a source selected from the group consisting of: ~~a cell, fetal cell, tissue, blood, serum, plasma, saliva, urine, tear, vaginal secretion, umbilical cord blood, chorionic villi, amniotic fluid, embryonic tissue,~~ lymph fluid, cerebrospinal fluid, mucosa secretion, peritoneal fluid, ascitic fluid, fecal matter, and body exudates.

Claim 91 (original): The method of claim 90, wherein said sample is blood.

Claim 92 (original): The method of claim 91, wherein said blood is from a pregnant female.

Claim 93 (original): The method of claim 92, wherein said blood is obtained from a human pregnant female when the fetus is at a gestational age selected from the group consisting of: 0-4, 4-8, 8-12, 12-16, 16-20, 20-24, 24-28, 28-32, 32-36, 36-40, 40-44, 44-48, 48-52, and more than 52 weeks.

Claim 94 (original): The method of claim 93, wherein said sample is obtained from plasma from said blood.

Claim 95 (original): The method of claim 87, wherein said agent is a cell lysis inhibitor.

Claim 96 (original): The method of claim 87, wherein said cell lysis inhibitor is selected from the group consisting of glutaraldehyde, derivatives of glutaraldehyde, formaldehyde, formalin, and derivatives of formaldehyde.

Claim 97 (original): The method of claim 96, wherein said cell lysis inhibitor is formalin.

Claim 98 (original): The method of claim 97, wherein the final concentration of formalin in the sample is selected from the group consisting of: 0.0001-0.03%, 0.03-0.05%, 0.05-0.08%, 0.08-0.1%, 0.1-0.3%, 0.3-0.5%, 0.5-0.7%, 0.7-0.9%, 0.9-1.2%, 1.2-1.5%, 1.5-2%, and 2-3%.

Claim 99 (original): The method of claim 98, wherein the final concentration of formalin in the sample is 0.1%.

Claim 100 (original): The method of claim 87, wherein isolation of nucleic acid comprises a centrifugation step.

Claim 101 (original): The method of claim 100, wherein the centrifugation step is performed with the centrifuge braking power set to zero.

Claim 102 (original): The method of claim 100, wherein the centrifugation step is performed at a speed selected from the group consisting of 0-50 rpm, 50-100 rpm, 100-200 rpm, 200-300 rpm, 300-400 rpm, 400-500 rpm, 500-600 rpm, 600-700 rpm, 700-800 rpm, 800-900 rpm, 900-1000 rpm, 1000-2000 rpm, 2000-3000 rpm, 3000-4000 rpm, 4000-5000 rpm, 5000-6000 rpm, 6000-7000 rpm, 7000-8000 rpm, and greater than 8000 rpm.

Claims 103-131 (cancelled)

Claim 132 (currently amended): The method of claim 1, wherein said sequence is determined by a method comprising:

- (1) amplification of the locus of interest;
- (2) exonuclease treatment of the products of (1);
- (3) single stranded DNA of (2) is annealed to an oligonucleotide to form an annealed template and primer;
- (4) incorporation of a nucleotide using the annealed template and primer of (3);
- (5) detection of the incorporated nucleotide.

Claim 133 (currently amended): The method of claim 58, wherein said sequence is determined by a method comprising:

- (1) amplification of the locus of interest;
- (2) exonuclease treatment of the products of (1);
- (3) single stranded DNA of (2) is annealed to an oligonucleotide to form an annealed template and primer;
- (4) incorporation of a nucleotide using the annealed template and primer of (3);
- (5) detection of the incorporated nucleotide.

Claim 134 (original): The method of claim 132 or 133, wherein the amplification method is selected from the group consisting of: polymerase chain reaction, self-sustained sequence reaction, ligase chain reaction, rapid amplification of cDNA ends, polymerase chain reaction and ligase chain reaction, Q-beta phage amplification, strand displacement amplification, and splice overlap extension polymerase chain reaction.

Claim 135 (original): The method of claim 134, wherein said method of amplification is by PCR.

Claim 136 (original): The method of claim 132 or 133, wherein said primer hybridizes adjacent to the locus of interest.

Claim 137 (original): The method of claim 132 or 133, wherein said incorporated nucleotide is a dideoxynucleotide or deoxynucleotide.

Claim 138 (original): The method of claim 132 or 133, wherein said incorporation reaction comprises two terminating nucleotides and two non-terminating nucleotides.

Claim 139 (original): The method of claim 137, wherein said incorporated nucleotide is labeled with a molecule selected from the group consisting of radioactive molecule, fluorescent molecule, antibody, antibody fragment, hapten, carbohydrate, biotin, derivative of biotin, phosphorescent moiety, luminescent moiety, electrochemiluminescent moiety, chromatic moiety, and moiety having a detectable electron spin resonance, electrical capacitance, dielectric constant and electrical conductivity.

Claim 140 (original): The method of claim 138, wherein said terminating nucleotides are labeled with a molecule selected from the group consisting of radioactive molecule, fluorescent molecule, antibody, antibody fragment, hapten, carbohydrate, biotin, derivative of biotin, phosphorescent moiety, luminescent moiety, electrochemiluminescent moiety, chromatic moiety, and moiety having a detectable electron spin resonance, electrical capacitance, dielectric constant and electrical conductivity.

Claim 141 (original): The method of claim 139, wherein the labeled nucleotide is labeled with a fluorescent molecule.

Claim 142 (original): The method of claim 140, wherein the terminating nucleotides are labeled with a fluorescent molecule.

Claim 143 (original): The method of claim 1, wherein said sequence is determined by a method comprising:

(1) amplification of the locus of interest, wherein the amplification reaction comprises a forward primer, a reverse primer, and a probe that anneals to the locus of interest, which is within the region of the amplicon; and

(2) detection of the PCR products, wherein the amount of PCR product is used to determine the presence or absence of a specific genetic sequence.

Claim 144 (original): The method of claim 58, wherein said sequence is determined by a method comprising:

(1) amplification of the locus of interest, wherein the amplification reaction comprises a forward primer, a reverse primer, and a probe that anneals to the locus of interest, which is within the region of the amplicon; and

(2) detection of the PCR products, wherein the amount of PCR product is used to determine the presence or absence of a specific genetic sequence.

Claim 145 (original): The method of claim 143 or 144, wherein the amplification is by PCR.

Claim 146 (original): The method of claim 143 or 144, wherein the probe contains a reporter dye at the 5' end and the 3' end contains a quenching dye.

Claim 147 (cancelled)

Claim 148 (currently amended): The method of claims ~~103, 104, 108, 109, 116, 117, 118, 119, 123, 124, 132~~ or ~~[[133,]]~~ 143 ~~or~~ 144, wherein an agent that inhibits cell lysis has been added to the sample to inhibit the lysis of cells, if present, and wherein said agent is selected from the group consisting of membrane stabilizer, cross-linker, and cell lysis inhibitor.

Claim 149 (currently amended): The method of claim ~~[[141]]~~ 148, wherein said agent is a cell lysis inhibitor.

Claim 150 (currently amended): The method of claim ~~[[141]]~~149, wherein said cell lysis inhibitor is formalin at a percentage selected from the group consisting of: 0.0001-0.03%, 0.03-0.05%, 0.05-0.08%, 0.08-0.1%, 0.1-0.3%, 0.3-0.5%, 0.5-0.7%, 0.7-0.9%, 0.9-1.2%, 1.2-1.5%, 1.5-2%, and 2-3%.

Claim 151 (currently amended): The method of claim ~~[[142]]~~150, wherein the concentration of formalin in the sample is 0.1%.

Claim 152 (currently amended): A method for detecting the presence or absence of a fetal chromosomal abnormality, said method comprising:

(a) determining the sequence of alleles of a locus of interest from template DNA, wherein the template DNA comprises a mixture of fetal DNA and maternal DNA, and wherein the template DNA is from a sample from a pregnant female,

(b) quantitating the relative amount of the alleles at a heterozygous locus of interest that was identified from the locus of interest of (a), wherein said relative amount is expressed as a ratio, and wherein said ratio indicates the presence or absence of a fetal chromosomal abnormality.

Claim 153 (withdrawn): A composition comprising fetal DNA and maternal DNA, wherein the percentage of free fetal DNA in the total free DNA of the composition is selected from the group consisting of: about 15-16% fetal DNA, about 16-17% fetal DNA, about 17-18% fetal DNA, about 18-19% fetal DNA, about 19-20% fetal DNA, about 20-21% fetal DNA, about 21-22% fetal DNA, about 22-23% fetal DNA, about 23-24% fetal DNA, about 24-25% fetal DNA, about 25-35% fetal DNA, about 35-45% fetal DNA, about 45-55% fetal DNA, about 55-65% fetal DNA, about 65-75% fetal DNA, about 75-85% fetal DNA, about 85-90% fetal DNA, about 90-91% fetal DNA, about 91-92% fetal DNA, about 92-93% fetal DNA, about 93-94% fetal DNA, about 94-95% fetal DNA, about 95-96% fetal DNA, about 96-97% fetal DNA, about 97-98% fetal DNA, about 98-99% fetal DNA, and about 99-99.7% fetal DNA.

Claim 154 (withdrawn): A composition comprising fetal DNA and maternal DNA, wherein the percentage of free fetal DNA in the total free DNA of the composition is selected from the group consisting of: about 15-16% fetal DNA, about 16-17% fetal DNA, about 17-18% fetal DNA, about 18-19% fetal DNA, about 19-20% fetal DNA, about 20-21% fetal DNA, about 21-22% fetal DNA, about 22-23% fetal DNA, about 23-24% fetal DNA, about 24-25% fetal DNA, about 25-35% fetal DNA, about 35-45% fetal DNA, about 45-55% fetal DNA, about 55-65% fetal DNA, about 65-75% fetal DNA, about 75-85% fetal DNA, about 85-90% fetal DNA, about 90-91% fetal DNA, about 91-92% fetal DNA, about 92-93% fetal DNA, about 93-94% fetal DNA, and about 94-95% fetal DNA.

Claim 155 (withdrawn): A prenatal diagnostic method comprising analyzing a composition comprising fetal DNA and maternal DNA, wherein the percentage of free fetal DNA in the total free DNA of the composition is selected from the group consisting of: about 15-16% fetal DNA, about 16-17% fetal DNA, about 17-18% fetal DNA, about 18-19% fetal DNA, about 19-20% fetal DNA, about 20-21% fetal DNA, about 21-22% fetal DNA, about 22-23% fetal DNA, about 23-24% fetal DNA, about 24-25% fetal DNA, about 25-35% fetal DNA, about 35-45% fetal DNA, about 45-55% fetal DNA, about 55-65% fetal DNA, about 65-75% fetal DNA, about 75-85% fetal DNA, about 85-90% fetal DNA, about 90-91% fetal DNA, about 91-92% fetal DNA, about 92-93% fetal DNA, about 93-94% fetal DNA, and about 94-95% fetal DNA.

Claims 156 - 180 (cancelled)

Claim 181 (new): The method of claim 1, wherein the sample is selected from the group consisting of: blood, serum, plasma, urine, and vaginal secretion.

Claim 182 (new): The method of claim 181, wherein the sample is blood.

Claim 183 (new): The method of claim 182, wherein the template DNA is obtained from plasma from said blood.

Claim 184 (new): The method of claim 182, wherein the template DNA is obtained from serum from said blood.

Claim 185 (new): The method of claim 8, wherein template DNA from said human pregnant female is obtained from a sample selected from the group consisting of: blood, serum, plasma, urine, and vaginal secretion.

Claim 186 (new): The method of claim 185, wherein the sample is blood.

Claim 187 (new): The method of claim 186, wherein the template DNA is obtained from plasma from said blood.

Claim 188 (new): The method of claim 186, wherein the template DNA is obtained from serum from said blood.

Claim 189 (new): The method of claim 11, wherein said cell lysis inhibitor is selected from glutaraldehyde, formaldehyde and formalin.

Claim 190 (new): The method of claim 58, wherein the sample was obtained from a pregnant female.

Claim 191 (new): The method of claim 190, wherein the pregnant female is human.

Claim 192 (new): The method of claim 191, wherein said sample is selected from the group consisting of: blood, serum, plasma, urine, and vaginal secretion.

Claim 193 (new): The method of claim 192, wherein said sample is blood.

Claim 194 (new): The method of claim 193, wherein the free fetal DNA is obtained from plasma from said blood.

Claim 195 (new): The method of claim 193, wherein the free fetal DNA is obtained from serum from said blood.

Claim 196 (new): The method of claim 63, wherein said cell lysis inhibitor is selected from glutaraldehyde, formaldehyde and formalin.

Claim 197 (new): The method of claim 86, wherein the sample is selected from the group consisting of blood, serum, plasma, urine, and vaginal secretion.

Claim 198 (new): The method of claim 197, wherein the sample is blood.

Claim 199 (new): The method of claim 198, wherein the template DNA is obtained from serum of said blood.

Claim 200 (new): The method of claim 198, wherein the template DNA is obtained from plasma of said blood.

Claim 201 (new): The method of claim 96, wherein said cell lysis inhibitor is selected from the group consisting of glutaraldehyde, formaldehyde, and formalin.

Claim 202 (new): The method of claim 152, wherein the sample is selected from the group consisting of: blood, serum, plasma, urine, and vaginal secretion.

Claim 203 (new): The method of claim 202, wherein the sample is blood.

Claim 204 (new): The method of claim 203, wherein the template DNA is obtained from serum from a blood sample from said female.

Claim 205 (new): The method of claim 203, wherein the template DNA is obtained from plasma from a blood sample from said female.

Claim 206 (new): The method of claim 133 or 144, wherein said agent is a cell lysis inhibitor.

Claim 207 (new): The method of claim 1 or 152, wherein said mixture comprises at least about 15% fetal DNA.

Claim 208 (new): The method of claim 1 or 152, wherein said mixture comprises a maximum of about 98-99% fetal DNA.